

AD-A222 345

12 REPORT SECURITY CLASSIFICATION (U)		11	
2a SECURITY CLASSIFICATION AUTHORITY N/A		3 DISTRIBUTION/AVAILABILITY OF REPORT Distribution unlimited	
2b DECLASSIFICATION/DOWNGRADING SCHEDULE N/A		5 MONITORING ORGANIZATION REPORT NUMBER(S) N/A	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) University of Maryland, College Park		6a NAME OF PERFORMING ORGANIZATION University of Maryland	
6b OFFICE SYMBOL (If applicable) N/A		7a NAME OF MONITORING ORGANIZATION Office of Naval Research	
6c ADDRESS (City, State, and ZIP Code) Department of Chemistry & Biochemistry College Park, Maryland 20742		7b ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, Virginia 22217-5000	
8a NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research		8b OFFICE SYMBOL (If applicable) ONR	
8c ADDRESS (City, State, and ZIP Code) 800 North Quincy Street Arlington, Virginia 22217-5000		9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-88-K-0323	
10 SOURCE OF FUNDING NUMBERS		10	
PROGRAM ELEMENT NO. 61153N		PROJECT NO. 1141MB	
TASK NO. 441N009-01		WORK UNIT ACCESSION NO.	
11 TITLE (Include Security Classification) Macromolecular Calculations for the XTAL-System of Crystallographic Programs			
12 PERSONAL AUTHOR(S) Stewart, James M.			
13a TYPE OF REPORT Annual		13b TIME COVERED FROM 6/1/89 TO 5/31/90	
14 DATE OF REPORT (Year, Month, Day) 1990, May, 24		15 PAGE COUNT 4	
16 SUPPLEMENTARY NOTATION Prepared in collaboration with K.B. Ward and D.M. Collins at the Naval Research Laboratory (Code 6030). See attached summary and preprints.			
17 COSATI CODES		18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	
19 ABSTRACT (Continue on reverse if necessary and identify by block number) During the last year effort has been concentrated on the completion of two programs to carry out least-squares refinement of macromolecules which preserve canonical stereochemistry. In addition, two other programs were completed, documented and placed in the XTAL system. In the coming year testing, debugging, and documentation will continue and a new program for crystallographic phase determination by maximum entropy methods will be written and tested. In addition three new links which should speed up the least-squares refinement process by approximately two orders of magnitude will be developed.			
20 DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21 ABSTRACT SECURITY CLASSIFICATION (U)	
22a NAME OF RESPONSIBLE INDIVIDUAL Dr. Michael Marron		22b TELEPHONE (Include Area Code) 202-696-4760	
		22c OFFICE SYMBOL ONR	

DATE: 22 May 1990

ANNUAL REPORT ON CONTRACT ONR N00014-88-K-0323

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CONTRACTOR: University of Maryland - Code 07419

CONTRACT TITLE: Macromolecular Calculations for the XTAL
System of Crystallographic Computer Programs

CONTRACT PERIOD: 1988 June 1 through 1991 May 31

RESEARCH OBJECTIVE: To produce, within the XTAL system of crystallographic computer programs, codes, documentation and tests for macromolecular crystallographic calculations.

This project is being carried out in collaboration with K. B. Ward and D. M. Collins of the Naval Research Laboratory (Code 6030).

During the last year, the implementation and documentation of least-squares refinement programs for macromolecular crystals has been completed. The documentation which has been generated for programs completed during the 1989/1990 year is included in the XTAL version 3.0 manual which will be distributed in September 1990. Preprints are attached to this report. The following brief description indicates the nature of the six programs which have been completed and documented and for which a simulated data test case has been prepared:

PROTIN: A program to read "constraint information" to describe the elements of a macromolecular crystal structure. This information, for example, can be the idealized coordinates for all the amino acids. This program is a translation of the Hendrickson-Konnert program by the same name. The purpose of this program when used in conjunction with PROLSQ is to preserve canonical stereochemistry.

PROLSQ: A program to do constrained least-squares refinement of the positional and thermal parameters of a macromolecular structure. This program is a translation into XTAL of the Konnert-Hendrickson program by the same name.

Two small service programs to support ongoing structure work were also written and documented, PHACMP and REFOUT. PHACMP allows a user to compare one reflection phase set with another on a statistical basis. REFOUT allows the user to transfer reflection information from the XTAL format to another in order to communicate with other programs available.

In addition to the programs PROTIN and PROLSQ prepared under this

PROTIN: Set Constraints for Refinement of Atomic Parameters

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SYNOPSIS

PROTIN combines the atomic parameters of the trial structure with canonical stereochemical information and sets up the constraint observational terms for PROLSQ the restrained-parameter structure-factor least-squares atomic parameter refinement program for large molecules.

This XTAL version is derived from the program of John H. Konnert (1976) which was elaborated for protein refinement by Wyane A. Hendrickson (1985). Since that time a number of others have made changes, in details, to the original code and documentation. Among these contributors are Barry Finzel, Steven Sheriff, Anita Sielecki, Janet Smith, & Alex Wlodawer.

PURPOSE

The pair of programs PROTIN and PROLSQ are designed to allow the refinement of the atomic parameters obtained from crystallographic diffraction data while restraining the parameters to conform to stereochemical information. These programs use stereochemical information as additional observations which are incorporated with the observations of reflection intensities so that the refinement of atomic parameters includes information in the observational equations about both stereochemistry and diffraction structure factors.

In the XTAL system PROATM is used to load the atomic parameters, identifying each atom in terms of its type and residue or group. PROTIN is used to load the canonical stereochemical information and connect the attributes of the ideal atoms to atoms of corresponding type and group in the given structure. Once the attachments are made, the quantities for the structure being refined which are needed to contribute to the matrix of normal equations are written to a file for use in the PROLSQ program. PROLSQ takes in this information and the diffraction information and forms and solves the combined least-squares normal equations for shifts in the atomic parameters.

Before using PROTIN/PROLSQ the Henderson paper (1985) should be read. Especially important are pages 264-268 where the "Refinement Strategy" is laid out.

METHOD

The method used in PROTIN/PROLSQ is documented in publications by Konnert and Hendrickson (1976,1979,1980,1985).
From the standpoint of use of these programs Hendrickson (1985) presents

an overview of the PROTIN-PROLSQ programs which are the ones which have been translated into RATHAC and placed in the XTAL system. A summary of this paper follows in which the input lines set forth below are related to the terms used in that paper.

In general the geometric data must be placed in the input stream in a very restricted order. Therefore, the input lines are grouped according to the kinds of stereochemical features which may be restrained.

The first lines PROTIN, SPCPEP, and ALTCMN set global conditions for the structure. SPCPEP, and ALTCMN are optional if the protein has well defined chains which have been specified in the ATOM lines loaded by PROATM. The PROATM program marks, through the lrsqu: record of the binary data file the N terminal and C terminal group of each chain. The chain identity and terminal residue information is then generated in PROTIN from the signals set by PROATM.

DISULB is optional if no disulfide linkages in the structure are to be restrained.

The optional lines from INTRAD to SYMMCT or the END line supplied at this point causes all the from RESTRM through ELRES, to be read from the "default" file, PRORES, which is supplied with the XTAL system. This file contains the images shown below. The groups contained in this file will serve for many substances. If there are other groups or restraints which must be defined it may be done either by editing the ascii PRORES file or by supplying all the lines shown below in the specified order.

NOTE WELL: That additional groups which are added must be added after those groups already present. As noted elsewhere in this document the line order is very restricted in PROTIN due to the structure of the program itself.

The lines, RESTRM, RESLWK, and RESIDU contain the canonical coordinates for the amino acids and some other well known moieties. They also serve to set up the character strings which identify groups and atoms within the groups. The substrings, TRM, LNK, and RES, are then used in forming names for lines which follow where the distinction between groups which are terminal on chains, linking in chains, or a residue in a chain is important in forming the restraint derivatives. The lines which follow use the strings established in the RESxxx lines to connect restraints to the defined groups. Thus any new groups added will require the addition of all the other corresponding restraint input lines described below.

DISTRM, DISLWK, and DISRES supply the connectivity for the groups. This permits the calculation of the important distances. Four types of distances have been distinguished: actual bond distances, the next-nearest-neighbor distances from the triple of atoms that define bond angles, first to fourth atom distances that relate to prescribed dihedral angles as within planar groups, and hydrogen bond lengths. In restraining these geometric factors all the information is encoded in terms of atom pair distances which are used to produce the factors in the normal equations formed in PROLSQ.

PLARES, PLAPRO, PLATRM, PLALNK and PLALNL serve to establish constraints on the coplanarity that exists and should be conserved in some groups. In PROTM/PROLSQ a method is used which restrains the deviations of the atoms of the refined structure from the least-squares plane of the group.

CHIRAL serves to define chiral centers within groups. The set of interatomic distances is insensitive to handedness so additional restraints must be imposed to assure the preservation of chirality. This is done by introducing a chiral volume equal to the triple scalar product of the vectors from a central atom to three attached atoms to quantify chirality. The sign of the chiral volume depends upon the handedness of the group and its magnitude equals the volume of the parallelepiped formed by the three vectors. The connectivity given in the CHIRAL line serves to allow the calculation of the ideal and model volumes to be used in forming the observational function.

VDMDIS serves to set up a table of atomic van der Waals diameters which may then be used in the calculations of non-bonded contacts.

VDMTRM, VDWLNL, VDWRES, serve to supply the number of contact distances characteristic of each group. VDMCON lines are used to supply the distance information in the form of pairs of atoms which might come in non-bonding contact within the group. In the case of these distances the constraints are designed to follow a potential energy function that feature a steep repulsive barrier against close contacts and a very weak attractive potential. The observational function used is taken only over possible repulsive contacts. That is when $d(\text{model}) < d(\text{minimum})$. The value of $d(\text{minimum})$ depends upon the atomic elements in contact and on the type of contact: single-torsion, separated atom pairs or multiple-torsion separated atoms.

TORTRM, TORRES, and their data supplying adjuncts NEIGHB, CHIDIS and CHIWGT serve to restrain torsion angles. Torsion angle restraints are among the least restricted of stereochemical features, but certain restrictions, related to non-bonded contacts, do apply. The nature of these restrictions, both for main-chain and for side-chains conformations in proteins are known. In PROTM a simple quadratic form for the torsional potential is used. The differences in the angle of the torsional potential minimum, χ_1 , is calculated for both for the ideal group and for the model and the difference in the square used in the observational function. The configurations treated can include quasi-planar torsion, as in the peptide bond, staggered potentials as in aliphatic side chains, transverse preferred conformations as in aromatic side chains, and targeted main-chain conformation angles such as in alpha helices. The definition of the torsion angles omega, phi, psi, $\chi_1(1)$, $\chi_1(2)$, etc. are given in Jane S. Richardson's paper (1981) which can serve as a useful general reference for the nomenclature used in setting up the restraints used in PROTM/PROLSQ.

SECTRM allows for the naming and characterizing of known secondary structural features in terms of characteristic χ_1 and phi values.

ELITRM and ELIRES serve to control the thermal parameters shifts by restraining the motion of bonded atoms relative to one another.

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INTERO, INTRAD, and SPCDIS restrain the distances between chains in structures with multiple chains.

SPCPLA serves to set up constraints for special planar groups.

EXCON serves to set up restraints which will exclude contacts between chains in the structure.

SECSL serves to set up restraints on elements of secondary structure for back-bone torsion-angles.

SPCSYM, SYMOP, and SYMWGT serve to set up constraints based on chains related by non-crystallographic symmetry.

The PROTM program should be run once before a series of refinements using PROLSQ. The augmenting normal-equation elements pertinent to the stereochemical restraints are stored in file P for communication to PROLSQ. The values in this file will change slowly as refinement takes place so that, in general, it is not necessary to rerun PROTM for every execution of PROLSQ. Of course after several cycles of least-squares or when additional atoms are added to the model, PROTM must be run again before PROLSQ.

NAMES OF CHAINS AND GROUPS/RESIDUES

In the description which follows a "chain identifier" will mean the character string supplied in field 5 of an ATOM line in PDB format or field 7 of a PROAT line. All reference to chains will be by these "names". Note that the PDB format limits chain names to 1 character while PROAT lines allow up to three characters. These names are placed sequentially in a table and transformed to relative pointers for use in PROTM and PROLSQ.

Groups are defined in the RESTRM, RESLNL, RESIDU and RESIDE lines. The symbols used for these groups must be used in the PROATM lines to identify the group to which an atom belongs. Residues are a special subset of these groups consisting of the amino acid groups which make up the chains. Each residue in a chain is assigned a "residue sequence number" during atom loading by PROATM. This number is the one in field 6 of a PDB ATOM line or in field 8 of a PROAT line. During processing these residue sequence numbers are compressed into a sequential set of pointers developed at atom loading. The user refers to his "residue sequence number" in the input lines which follow, but it is well to keep in mind, that internally these pointers will dominate. In this case error messages may give the pointer which will require thought to find which residue is being pointed to.

The term "order number" refers to the number which points to the relative position of a given atom in a group. For example, arginine, ARG contains atoms N, CA, C, O, CB, CG, CD, NE, CZ, NH1, and NH2 with order numbers 1 through 11. The order numbers of linking groups are sometimes negative as a signal that the atoms are in the previous residue. This term is used in the data lines which follow and may appear in error messages from PROTM.

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PRINTER OUTPUT

Only the essential counts of restraints and atoms are printed at priority 3. Printer output is copious at priority 4. At 4, all the input data is echoed, and the compound specific distances, angles, etc. displayed.

FILE ASSIGNMENTS

Reads atom and unit cell data from a BDF on file A
Writes geometric constraint data for PROLSQ on file F
Optionally reads standard constraint data from file PRORES on profile:

CONTROL LINES

NOTE: control line order is very restricted in PROLIN. In the following list certain control lines must be used many times in groups. Within the groups the use of one line requires the use of a following line or lines. The whole group is then repeated. The groups are set off in the sequence listing which follows. All groups must follow one another in the sequence shown below.

PROLIN Program initiation line

Group 1 input lines set up standard groups dictionary

*DISULB Identify disulfide bridges
*FIRSTP Identify atoms with population parameters to be refined
*SPQPEP Identify the C and N terminus groups for each chain
*ALTCEN Identify alternate chains

Note that, if at this point all the lines from RESTRM through ELLRES are left out the values will be taken from the PRORES file. This is the default file of idealized groups.

A PRORES file is supplied with the XTAL system which contains all the usual groups encountered in protein structures.

Group 2 input lines set up ideal coordinates of residues

*RESTRM Identify and idealize terminal and main residues
*RESLTK Identify and idealize linkage residues
*RESIDU Identify and idealize standard chain residues and other groups
*RESIDE Identify and idealize the last standard residue at ends of chains

Group 3 input lines set up distance restraints

*DISTRM Characterize terminal chain residue interatomic distances
*DISLTK Characterize linking chain residue interatomic distances
*DISRES Characterize normal chain residue interatomic distances

Group 4 input lines set up plane restraints

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*PLARES Identify planar groups in standard residues
*PLAYPRO Identify multi-planar prosthetic planar groups
*PLATRM Identify terminal planar groups
*PLALNK Set up the four linking planar groups
*PLALML Specify restraining pairs for linking planar groups

Group 5 input lines set up chiral center restraints

*CHIRAL Characterize ideal chiral centers

Group 6 input lines set up contact restraints

*VDMDIS Set van der Waals diameters by atomic species
*VDWTRM Number of non-bonded contacts for specified terminal residue
*VDWLTK Number of non-bonded contacts for specified linking residue
*VDWRES Number of non-bonded contacts for specified residue
*VDWCON van der Waals contact codes for VDWTRM, VDWLTK, or VDWRES

Group 7 input lines set up torsion angle restraints

*TORTRM Identify atoms defining ideal torsion angles in terminal residues
*NEIGHB Identify neighbors in torsion groups
*CHIDIS Set neighbor distance identification
*TORRES Identify atoms defining ideal torsion angles in chain residues
*CHWGTT Set weighting codes for torsion angles
*SECTR Characterize secondary structure features by psi and phi values

Group 8 input lines set up anisotropic thermal restraints

*ELLTRM Set ellipsoid specifications for terminal groups
*ELLRES Set ellipsoid specifications for main chain groups

Note that the following lines are optional. All references to chain identities and user residue numbers are in terms of the names and numbers supplied through the PROATH program. These names and numbers are translated to pointers for use in the calculations. Some error messages may display these pointers. In this case the chain pointers will be 1, 2, ... In order of chain identification encountered in the ATOM or PROAT lines loaded in PROATH. The residue pointers will be the sequence number for the residue in order of loading by PROATH.

Group 9 input lines set up special distances restraints

*INTERD Set up blocks of inter chain special distances
*INTRAD Set up blocks of intra chain special distances
*SPCDIS Set up special inter-group distances

Group 10 input lines set up special plane restraints

*SPCPLA Specify special planar groups

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Group 11 input lines set up contact exclusion restraints

*EXCON Specify distance identifiers for contact exclusion

Group 12 input lines set up backbone torsion angle restraints

*SECSSEL Specify elements of secondary structure for backbone

Group 13 input lines set up noncrystallographic symmetry restraints

*SPCSYM Identify chains related by noncrystallographic symmetry

*SYNPOP Rotation and translation of a noncrystallographic symmetry

*SYMWGT Set noncrystallographic symmetry restraint weighting specs

END One and only one END line required in input stream

PROTIN Program initiation line

PROTIN

Field 1	Field 2-	Function	Option code
1	Compound ID	Print atom coordinates	ATL
2	Signal neutron data	Signal neutron data	NEU
3	Limit for van der Waals distances	VDW x.x	CEL n
4	Expect hydrogen atoms	HAT	NAP n
5	Expect individual aniso t.f.	ANI	
6	Identify disulfide bridges	ATL	

DISULB

Field 1	Field 2	Function
1	Chain identifier of CYS residue	Identify disulfide bridges
2	Residue sequence number on one side of the S-S bridge.	Chain identifier of CYS residue
3	Chain identifier of CYS residue on other side of the S-S bridge.	Residue sequence number on that side.
4	Residue sequence number on the other side.	Chain identifier of CYS residue

FIRSTP

Identify atoms with population parameters to be refined

Field 1	Field 2	Function
1	Name of the first atom, in the notation of PROATM on loading, to have the population parameter refined. This atom and all atoms to the end of the atoms loaded will have their population parameters refined in the subsequent PROLSQ run.	Identify atoms with population parameters to be refined
2	Identify "cis" residues	Name of the first atom
3	Chain identifier symbol e.g. A2	Identify "cis" residues
4	Number of cis peptide bonds in chain	Chain identifier symbol
5	Residue sequence number of cis peptide	Number of cis peptide bonds in chain
6	Identify alternate chains	Residue sequence number of cis peptide

ALTCN

Field 1 Primary chain identifier for mothers of alternate chains

Field 2 Secondary chain identifier for mothers of alternate chains

if there is 1 chain and 1 alternate chain: 1 0

if there is 1 chain and 2 alternate chains: 1 1

if there are 2 chains and each has 1 alternate chain: 1 1

if there are 2 chains and only chain 1 has 1 alternate chain: 1 0

if there are 2 chains and only chain 2 has 1 alternate chain: 2 0

Alternate conformations of discretely disordered residues are assigned during atom loading a different chain number from the mother chain for a given residue. Only the atoms which are modeled in different positions should be included among the input atoms.

Set up groups (residues) in ideal form as a dictionary

RESTRM
RESLKN
RESIDU
RESIDE

Terminal
Linking
"Standard"

Note last of "standard" groups

Field 1 Group name for residue e.g. NAMINO ; ASP ; HIS

Field 2 Name of atom in the group e.g. CA for alpha carbon

Field 3 X Cartesian coordinate in angstroms relative to C alpha as the origin in each group

Field 4 Y Cartesian coordinate

Field 5 Z Cartesian coordinate

Field 6 Order number of atom within the group e.g. 1 for N, 2 for C alpha, etc. For all standard groups, save glycine, this number starts at 5 because the coordinates of RESTRM MAIN are automatically taken for the first four atoms of every group. This number may also be negative in RESLKN lines in order to designate atoms belonging to a "previous" residue.

Field 7 Single letter code which is to be used as a synonym for the group name e.g. G for GLY. Used only for standard groups.

Set up distance restraints for idealized groups

DISTRM
DISLKN
DISRES

Field 1 Group name as defined in RESxxx input lines e.g. NAMINO

Field 2 Order number of origin atom for corresponding distance e.g. 1 for N, 2 for C alpha, etc.

Field 3 Order number of terminus atom for corresponding distance

Field 4 Code to define weight that is to be used to restrain distance

Field 5 Code to define weight that is to be used to restrain B values

Field 6 Code to define weight that is to be used to restrain B values

Field 7 Any distance for which B restraint is not required

1 Bond distance between two main chain atoms
 2 Angle distance between two main chain atoms
 3 Bond distance involving at least one side chain atom
 4 Angle distance involving a hydrogen
 5 Bond distance involving a hydrogen
 6 Angle distance involving a hydrogen
 Fields 6-9, 10-13, etc. as for Fields 2-5. Note that lines may be continued by placing a "C" as the last field on a line. The next line must then have the same ID for both line type and group name. Fields will continue in groups of four to complete the required restraints.

Set up restraints on planarity for idealized groups

PLAPRO
 PLATRN
 PLALNK

Field 1 Group name
 2- The order number of the atoms of the group which are to be restrained to planarity. If a group has no planes fields 2- are left blank.

Field 1- Codes specifying all the possible "restraining pairs" among the atoms specified in the RESLAK input lines. Note that only the number of atoms specified to be in planes and given in the PROTIN line under directive NAP will be considered to restrain the plane.

Set up chiral centers for idealized groups

CHIRAL

Field 1 Group name
 2 Descriptor code for how the chirality of the group is defined
 1 for groups "intrinsically" chiral
 0 for chirality related to nomenclature e.g. LEU VAL

Set up contact distances for idealized groups

VDWDIS

Field 1 Name of atom species e.g. HYDROGEN (limit 12 characters)
 2 Atomic number of species or atomic scattering factor pointer
 3 Carbonyl is arbitrarily set to 3
 van der Waals diameter of the species or distance between centers of two non-bonded atoms of the species

VDWTRM
 VDWRES

Field 1 group name
 2 number of non-bonded contacts for group

VDWLK

Field 1 group name

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2 number of non-bonded contacts for group
 3 number of non-bonded contacts specified for prolyl link
 These lines follow their corresponding VDWRES, VDWTRM, or VDWLK lines. There must be enough of these lines to satisfy the counts given in the header lines.

VDMCON

Field 1 Group name
 2 Order number of origin atom corresponding to a possible non-bonded contact
 3 order number of terminus atom for corresponding possible non-bonded contact
 4 Non-bonded distance restraint class as:

1 to indicate that the relative position of the given atoms is determined by only one torsion angle
 2 to indicate that the relative position of the given atoms is determined by two or more torsion angles
 5-7, 8-10, etc. as 3-4 continuing onto additional lines as required to satisfy the counts given in the preceding VDWRES, VDWTRM, or VDWLK lines.

Set up torsion angle restraints for idealized groups

TORTRM
 TORRES

Field 1 Group
 2 Atom order number for C(i-1)
 3 Atom order number for N(i)
 4 Atom order number for C alpha(i)
 5 Atom order number for C(i)
 6 Atom order number for N(i+1)
 7 Atom order number for C alpha(i+1)
 fields after 7 are ignored in TORTRM lines
 8 Atom order number for C beta(i)
 9 Atom order number for C gamma(i)
 10 Atom order number for C delta(i)
 11 Atom order number for C epsilon(i)
 12 Atom order number for C zeta(i)
 13 Atom order number for N ?(i)
 C(i-1)-N(i)-C alpha(i)-C(i)
 N(i)-C alpha(i)-C(i)-N(i+1)
 C alpha(i)-C(i)-N(i+1)-C alpha(i+1)
 N(i)-C alpha(i)-C beta(i)-C gamma(i)
 C alpha(i)-C beta(i)-C gamma(i)-C delta(i)
 specifies phi
 specifies psi
 specifies omega
 specifies chi(1)
 specifies chi(2)

NEIGHB

Field 1 Group
 2- Neighbor identification codes
 -1 for atoms from residue i-1
 0 for atoms from residue i
 1 for atoms from residue i+1
 5 for atoms from terminal groups e.g. OT of carboxyl terminus

CHIDIS

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Field 1 Group
2 Distance identification codes as specified in field 4
of the DISRES input line. Negative numbers imply ??????

CHIMCT Field 1 Group
2- Weighting code for side chain chi angles
0 for no specifications e.g. chi(5) of ARG
2 for planar e.g. aliphatics
3 for staggered e.g. chi(2) of aromatics
4 for orthonormal

SECSTR Field 1 Name for element of structure up to 20 characters maximum
2 code for secondary structure element
1 alpha helix
2 3(10) helix
3 pi helix
4 collagen type helix
5 parallel beta sheet
6 anti-parallel beta sheet
7 classic beta bulge position 1
8 classic beta bulge position 2
9 beta bend [or turn] type I position 2
10 beta bend [or turn] type I position 3
11 beta bend [or turn] type II position 2
12 beta bend [or turn] type II position 3
13 gamma turn position 1
14 gamma turn position 2
15 gamma turn position 3
3 Characteristic phi value
4 Characteristic psi value
5 Literature reference of up to 40 characters

Set ellipsoid specifications

ELLTRM Field 1 Group name
ELLRES 2 order number for atom for which ellipsoid is being specified
3 order number for atom p(0)
4 order number for atom p(1)
5 order number for atom s(0)
6 order number for atom s(1)
Note that negative numbers are used to point to atoms in preceding residues.
7-11, 12-16, etc. for additional ellipsoid atoms; new lines to continue.

INTRAD Set intra and inter chain distance restraints

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Field 1 Origin chain identifier

INTERD Field 1 Origin chain identifier
2 Target chain identifier

There must be SPCDIS lines following each INTRAD or INTERD line supplied. The number required will depend upon the number of special inter-group distances for this chain block to be restrained. e.g. S gamma - S gamma from two different CYS groups that form a bridge; distance from solvent to protein; etc. One line per pair of atoms for which distance is to be restrained.

SPCDIS Field 1 Residue sequence number of group to which origin atom belongs
2 Order number of origin atom within group
3 Residue sequence number of group to which target atom belongs
4 Order number of target atom
5 Distance in Angstroms to which the distance between the atoms should be restrained
6 Code to determine weight that should be used to restrain distances. Same as those shown for DISRES lines.
7 Code to determine weight that should be used to restrain temperature factors. Same as those shown for DISRES lines.

Specify special planar groups

SPCPLA Field 1 Chain identifier of first atom in special plane
2 Residue sequence number of first atom in special plane
3 Order number of first atom in special plane
4-6 The same quantities as in 1-3 for the second,
7-9 third, ... atoms which define the special plane.
etc.

Specify contact exclusion

EXCON Field 1 Chain identifier of first atom for which contacts are to be excluded
2 Residue sequence number of first atom
3 Order number of first atom
4-6 The same quantities as in 1-3 for the second, atom which defines the contact exclusion.

Specify elements of secondary structure for the backbone

SECSSEL Field 1 Chain identity; all chains of this type will be set
2 Residue sequence number of the initial group for this region of structure
3 Residue sequence number of the final group for this region of structure
4 Code for structure type

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-1 restrain phi and psi values to those of initial structure
 0 no restraints on phi and psi
 n restrain psi and phi values specified in SECSTR line
 for this n

Specify special non-crystallographic symmetry

SPCSYM Field 1-n Chain identities for chains in this special
 non-crystallographic symmetry group
 Each SPCSYM line must be followed by one or more SYMOP lines.

SYMOP Field 1-12 R11, R12, R13, T1, R21, R22, R23, T2, R31, R32, R33, T3

SYMGMT Field 1 Initial residue sequence number to which the SYMOP applies
 2 Final residue sequence number to which the SYMOP applies
 3 weighting code

Code	Main chain	Side chain
1	1	1
2	1	2
3	1	3
4	2	2
5	2	3
6	3	3

4-6, 7-9,... as in 1-3 for all group spans specified
 Where 1=tight; 2=medium; 3=loose

EXAMPLE

Note that the program PROATM must have been run before PROTON may be run
 in order that the atom coordinates to be constrained will be available on
 file A so that the appropriate constraint information can be generated
 on file P for use in the refinement process.

TITLE CRAMBIN FROM ARABIDOPSIS CABBAGE SEED-HENDRICKSON & TEETER 4 APR 89 JMS.
 PROTON CRAMB VDW 4.51 NAP 5 ATL GNL CEL 3 ANISO

END
 PROLSQ CRAMB NCY 25 PCH ISO
 PRINT ALIS 1
 STATS 5.0 4.0 3.0 2.0 1.0
 RTEST 5 23 1 1 4 3 .25 .45 .65 1.25 .35 .65 .95
 END
 FINISH

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PROLSQ: Constrained Refinement of Atomic Parameters

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SYNOPSIS

PROLSQ is a restrained-parameter structure-factor least-squares atomic
 parameter refinement program for large molecules. This XTAL version is
 derived from the program of John H. Konnert (1976) which was elaborated for
 protein refinement by Wayne A. Hendrickson (1980). Hendrickson (1985) has
 described the technique and philosophy of the use of PROLSQ in a readily
 acquirable publication.

Since that time a number of others have made changes, in details, to the
 original code and documentation. Among these contributors are Barry Finzel,
 Steven Sheriff, Anita Sielecki, Janet Smith, & Alex Wlodawer.

PURPOSE

The program will refine atomic models using both the conditional structure-factor least-squares procedure described by Waser and geometric restraints so that the canonical stereochemistry of the sub-elements of the structure will be taken into account in the least-squares process.

The information about the restraining geometry must be supplied by use of the PROLIN program of Hendrickson which supplies the geometry in terms of connectivity, covalent radii, bond angles, planarity, chirality, and other structural features of macromolecular substances. This information and the diffraction data are combined in the derivative matrix of the least-squares procedure such that elastic bounds are imposed on the geometry of the model. The restrained least-squares procedure uses a greatly reduced, "sparse", matrix. The parameter shifts are derived from this matrix not by inversion but by the iterative conjugant gradient method of Hestenes & Stiefel applied, described, and programmed by Konert (1976).

This approach has to refinement several advantages over conventional structure factor least-squares procedures: first, the size of the matrix increases linearly rather than quadratically. Second, the restraints effectively reduce the number of parameters to be determined. Third, the method usually shows a larger radius of convergence. Fourth, all parameters may be refined simultaneously, but only correlations dictated by the geometry are included in the derivative matrix.

Before the PROLIN/PROLSQ programs are used, Hendrickson's paper (1985) should be read. In the section "Refinement Strategy", pages 264-268, is given an outline of the appropriate use of these codes for the refinement of macromolecular structures.

METHOD

The method is described by Konert (1976). The function minimized is the weighted sums of the difference in the squared F calculated and F relative, of squared ideal and observed interatomic distances, and of other types of restraints. The list of the currently allowed restraints is given in the documentation of PROLIN and will not be reiterated here. The weighted, squared differences are multiplied by appropriate numerical differentials of the structure factors, the distances, or other factors, with respect to the parameters of the structure to form the vector of the normal equations. The matrix of the normal equations is formed from the derivative terms alone. Only correlations between atoms related by constraining features of the structure are saved in memory. This results in the formation of a "sparse" matrix since the off-diagonal elements are dispersed throughout the matrix. Since all parameters are refined simultaneously and standard matrix inversion methods are not space efficient and the problem is not separable into a number of smaller sets of equations, as in conventional block-diagonal least squares Konert uses the conjugate gradient iterative technique for the solution of the normal equations. The conjugant gradient technique retains the sparse derivative matrix throughout the solution for the shifts in atomic parameters. The non-zero elements of the hypothetical n by n derivative

matrix are retained during the iterative solution procedure. For the refinement of a structure of n atoms and m distance restraints, for example, the number of related elements in one half of the symmetric matrix that need be stored is $6n + 9m$. Since m is roughly linear with n , m is about $1/3n$, storage space required varies linearly with n . To ensure rapid convergence, it is necessary to determine the optimum damping factor for the computed shifts. In the XTAL version of PROLSQ this value is set to 0.5 by default. However, use of the TEST line will permit calculating R for a selected sub-set of reflections. Once a suitable damping factor is found it must be applied to all shifts if the desired geometry is to be retained.

REFINEMENT WEIGHTS

The optional WGTXXX input lines allow for the adjustment of refinement weights. These weights are given in terms of "standard deviations" of the class of observation. The default values set in this XTAL version are similar to those shown by Hendrickson (1985). In this paper there is a section devoted to the proper use of weights.

The setting of these weights is very important to the successful use of PROLSQ on two accounts. First, if the restraint weights are imbalanced with respect to geometric restraints and structure factor refinement, one will dominate the other giving rise to large divergence from ideal geometry or divergence in terms of the structure factors. If the restraints are too heavily weighted the R value may actually increase as the refinement goes forward since the conformity to the idealized model will dominate the structure factor information. Second, if any of the weights cause the weighted deltas squared to be very large there is the possibility that "floating point overflow" will occur during the conjugant gradient process, especially on 32 bit machines.

The numbers in the output which should be monitored are the "average weighted delta squared" values for each of the restraints and for the structure factors. In a reasonable refinement these values all approach a common value, usually near 1.0 as the refinement proceeds to completion. Furthermore, if any of the categories shows a value much larger than the others, then that restraint will apply with the greatest effect in the least-squares process.

The values of the weights may be set by the use of the WGTXXX input lines and by the inclusion of experimental sigma F values. See Hendrickson's paper for elaboration of this point. A value for the weights that will force the average to 1.0 may be found by looking at the corresponding value of the average delta given in the output and supplying this value as the sigma in the appropriate WGTXXX line.

For example consider the following "snapshot" of input and output:

Including the input lines:
WGTB *2 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5

Produces the following output:

[illegible]

```
SUM(WGT*DELTA**2) = .4163904+04
AVG(WGT*DELTA**2) = 23.525 FOR ISOTROPIC THERMAL FACTORS
```

TYPE	ROOT 1/WGT	NUMBER	RMS DELTA	SIGMA	MEAN DELTA	RMS DELTA	U	STGMA	THERMAL FACTOR TYPE
1	.500	21	1.912	.500	1.296	.1281	.07	MAIN-CHAIN BOND	
2	.500	21	2.278	.500	1.676	.1457	.07	MAIN-CHAIN ANGLE	
3	.500	42	2.487	.500	1.575	.1412	.07	SIDE-CHAIN BOND	
4	.500	63	3.060	.500	1.947	.1570	.07	SIDE-CHAIN ANGLE	
5	.500	0	.000	.500	.000	.0000	.07	X-H BOND	
6	.500	0	.000	.500	.000	.0000	.07	X-H ANGLE	
7	.500	0	.000	.500	.000	.0000	.07	METAL COORD. ETC.	
8	.500	3	1.342	.500	1.341	.1303	.07	HYDROGEN BOND	

AGREEMENT FACTORS BASED ON PARAMETERS BEFORE THIS CYCLE

$$\text{SUM(WGT*DELTA**2)} = .5221144+07 \quad \text{AVG(WGT*DELTA**2)} = 1508.57$$

	NUMERATOR	DENOMINATOR	R
STANDARD R-FACTOR	3203.9	68627.7	.047
WEIGHTED R-FACTOR	2285.0	4'805.6	.049
ERROR R-FACTOR	686.3	68627.7	.010

USED	REJECTED	R SURVEY	R SURVEY
3461	0	0	0

93 IS THE AVERAGE \FO-FC\ DISCREPANCY

----- RESOLUTION BREAKDOWN -----			<SIGF>/USED	R SHELL	R SPHERE
DRIN	REFS	<SIGF>/DATA			
36	36	.72	3.43	.041	.041
5.00	36	.72	2.998	.050	.048
3.00	116	.73	2.79	.050	.048
2.50	101	.46	2.33	.044	.047
2.00	226	.39	1.90	.051	.048
1.70	277	.28	1.43	.043	.047
1.50	324	.22	1.02	.047	.047
1.30	2381	.12	.16	.046	.047
ALL	3461				.047

MODELED DELTA = (2.5) + (-10.0)*(S-1/6)

FITTED <FO-FC> = (2.5) + (-10.3)*(S-1/6)

Then, from the same starting point, the run is made again having altered only the weight line. Note the values of delta in the first run and the values of sigma in the second run:

```

Input:
WGTB *2 1.9 2.3 2.5 3.1 0.5 0.5 1.3
WGTREF 1 2.5 -10.0

```

Output:

[illegible]

```

SUM(WGT*DELTA**2) = .1480259+03
AVG(WGT*DELTA**2) = .836 FOR ISOTROPIC THERMAL FACTORS

```

TYPE	ROOT	RMS	MEAN	RMS	SIGMA	U	SIGMA
	1/WGT	DELTA	DELTA				
1	1.900	21	1.912	1.900	1.296	.15	MAIN-CHAIN BOND
2	2.300	21	2.278	2.300	1.676	.17	MAIN-CHAIN ANGLE
3	2.500	42	2.487	2.500	1.575	.17	SIDE-CHAIN ANGLE
4	3.100	63	3.060	3.100	1.947	.19	SIDE-CHAIN ANGLE
5	.500	0	.000	.500	.000	.07	X-H BOND
6	.500	0	.000	.500	.000	.07	X-H ANGLE
7	.500	0	.000	.500	.000	.07	METAL COORD. ETC.
8	1.300	3	1.342	1.300	1.341	.12	HYDROGEN BOND

AGREEMENT FACTORS BASED ON PARAMETERS BEFORE THIS CYCLE

```
SUM(WGT*DELTA**2) = .5221144+07  AVG(WGT*DELTA**2) = 1508.57
```

	NUMERATOR	DENOMINATOR	R
STANDARD R-FACTOR	3203.9	68627.7	.047
WEIGHTED R-FACTOR	2285.0	46805.6	.049
ERROR R-FACTOR	686.3	68627.7	.010

USED	REJECTED	R SURVEY	R SURVEY
3461	0	0	0

93 IS THE AVERAGE \FO-FC\ DISCREPANCY

----- RESOLUTION BREAKDOWN -----			<SIGF>/USED	R SHELL	R SPHERE
DMIN	REFS	<SIGF>/DATA			
5.00	36	.72	3.43	.041	.041
5.00	36	.72	2.998	.041	.041
5.00	36	.72	3.673	.048	.048
5.00	116	.73	2.79	.050	.050
2.50	101	.46	2.33	.044	.047
2.50	101	.46	2.008	.051	.048
2.00	226	.29	1.90	.048	.048
2.00	226	.29	1.977	.051	.048
1.70	277	.28	1.43	.043	.047
1.50	324	.12	1.02	.047	.047
1.30	324	.12	1.020	.047	.047
1.30	2181	.12	.568	.046	.047

ALL 3461

MODELED DELTA = (2.5) + (-10.0)*(S-1/6)

FITTED <FO-FC> = (2.5) + (-10.3)*(S-1/6)

ATOM NAMES IN LISTS

Each atom in the structure is loaded from the XTAL program PROATM. PROATM reads atom parameters and "names" in the Protein Data Bank format. The atom names consist of an atom serial number, which changes as the numbers of known atoms in the model changes; an atom name, which consists of a scattering factor symbol, a remoteness indicator code, and a branch designator; an alternate location indicator; a residue name; a chain identifier; a residue sequence number; and a code for insertion of residues. In PROLSQ listings these name parts are compressed to a 9 character string for compact listing purposes. This string is structured in the following way:

Position	Contents
1	An integer pointing to the chain to which the atom belongs
2	A single letter code for the residue to which the atom belongs
3-5	The residue sequence number from the PDB input to PROATM
6-9	The atom name; symbol, remoteness, and branch; left justified

The single letter codes used by PROLSQ are shown in the following table which shows the three character and one character code side by side.

Residue	3	1	Residue	3	1
Alanine	ALA A	Arginine	ARG R	Asparagine	ASN N
Aspartic acid	ASP D	ASP/ASN ambiguous	ASX B	Cysteine	CYS C
Cysteine	CYS C	Glutamine	GLN Q	Glutamic acid	GLU E
GLU/GLN ambiguous	GLX Z	Glycine	GLY G	Histidine	HIS H
Isoleucine	ILE I	Leucine	LEU L	Lysine	LYS K
Methionine	MET M	Phenylalanine	PHE F	Pro'line	PRO P
Serine	SER S	Threonine	THR T	Tryptophan	TRP W
Tyrosine	TYR Y	Valine	VAL V		

PRINTER OUTPUT

The total mass of printing may be controlled with the use of a PRINT line. The print line allows for selected and limited printing of shift, reflection, and selected large deviations from distance restraints at priority 3. Most echoing of constraint information is done at priority 4. The results of each cycle are printed at priority 3 as are R values which result from use of the RREST input line. If a sampling of the matrix and vector elements is desired, print priority 5 may be specified. This feature will result in copious printed output in all but the smallest test cases.

FILE ASSIGNMENTS

Reads atom and reflection data from a BDF on
Writes refined atomic parameters and Fc on
Scratches an intermediate BDF on
Reads geometric constraint data from PROTIN on
Optionally writes reflections excluded from refinement on
Optionally writes reflections for an R value survey on
Optionally writes images of Protein Data Bank ATOM lines on

file A
file B
file E
file F
file G
file H
unitpch:

CONTROL LINES

PROLSQ
*ARCHIV Program initiation line
*BOV Specify items to be placed in reflection records
*DAMPISH Overall isotropic temperature factor
*PRINT Shift damping factors to control refinement
*REFLIM Print control line
*RTEST Limits on reflections to be included in refinement
*SCALE Set up survey of R values to assess refinement
*SCANIS F relative scale factors
*SOLMOD Anisotropic scale factor
*STATS Solvent model parameters
*WGTHB Number and limits of d shells for R value survey
*WGTHCI Set thermal restraint weighting
*WGTHCON Set chiral center restraint weighting
*WGTHDIS Set contact distance restraint weighting
*WGTHPLA Set plane restraint weighting
*WGTHREF Set structure factor restraint weighting
*WGTHSHF Set excessive shift restraint weighting
*WGTHSYM Set noncrystallographic symmetry restraint weighting
*WGTHTOR Set torsion angle restraint weighting
*NOREF Control atoms included in refinement

PROLSQ	Field 1	compound ID	2- Function	Option code
			Congugent gradient cycles	NCY n <n=50>
			Lagrange multiplier scale	LAG n <n=10>
			Normal refinement	<REF>
			Ideal structure refinement	IDR
			no structure factor calc.	IDF
			Skip refinement and only	SKI
			calculate structure factors	IZE
			Evaluate idealization but do	ANO
			not refine	
			Apply anomalous dispersion	

do not apply dispersion	<NOA>	
Temperature factors type		
overall isotropic	OVA	
individual isotropic	ISO	
Data set number	DSN n	<n-1>
Constrain origin		
along x	ORX	<default is no>
along y	ORY	<default is no>
along z	ORZ	<default is no>
"Punch" atom coordinates		
in PDB format on unitpch:	PCH	<default is no>
Control items in lrrel: of output BDF		
Field 1 - ID numbers of data items to be added to or deleted from the BDF.		
Positive ID numbers signal addition, negative deletion. See long comment in program FC.		
Set overall isotropic temperature factor		
Field 1		
Either B overall or U overall depending on <B from input BDF> magnitude. If value is 0 < B < 0.2 the quantity is multiplied by 78.9568 and stored as B.		
If the quantity is 0.2 < B < 40.0 it is accepted as B and stored. The line is ignored unless in overall t.f. mode.		
Set parameter shift damping factors		
Damping factors to be applied to parameter shifts determined by the least-squares process. These factors will be used to establish the new parameters written to the output BDF on file B and, optionally on unitpch: Factors between 0 & 4 allowed.		
N.B. Unequal damping factors will distort the geometry.		
Field 1		<0.5>
Field 2		<0.5>
Field 3		<0.5>
Field 4		<0.5>
Field 5		<0.5>
Field 6		<0.5>
Field 7		<0.5>
Field 8		<0.5>
Field 9		<0.5>

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Field 10	Damping factor for B13 parameter shifts	<0.5>
Field 11	Damping factor for B23 parameter shifts	<0.5>
PRINT		
Field 1-	Control the volume of printed information	
Option control codes	Option code	
Function		
List first m reflections	LFIR m	<m=10>
List reflection data	FLIS m	<m=0> list every mth reflection
List first n input atoms	ILIS n	<n=99999>
ILIS not present means no input atoms listed		
List updated atoms	ALIS m	<m=2>
m = 0/1/2 for none/all/beyond shift limits set below		
Atom parameter shifts on x, y, and z where x.x is distance in A	PARC x.x	<x.x=0.1> only those shifts > x.x
temperature factor shifts	BCUT x.x	<x.x=0.25>
population parameter shifts	POPC x.x	<x.x=0.25>
Torsion angle printing		
all standards and statistics	STOR	
statistics only	<NTOR>	
single angle contacts	CTOR x.x	<x.x=0.1>
multiple angle contacts	MTOR x.x	<x.x=0.1>
Chiral centers printing		
worst case chiral centers	WCHI	
all chiral centers	ACHI	
statistics only	<NCHI>	
Hydrogen bond printing		
all possible H bonds	HBON	
statistics only	<NHBO>	
Restrained distances printing		
distances of type code "j"	DISC x.x	<x.x=5.0>
if		x.x=0.0 for print all
DIDEAL-DMODEL >= x*SIGD(J)		
Restrained planes printing		
all planes with RMS deviation > x*SIGP	PLAN x.x	<x.x=2.0>

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SCALE

Field 1 F relative scale factor <value from input BDF>
 2 Scale group number <1>

SCANIS

Set an overall anisotropic scale factor

Overall anisotropic scaling of F calculated

Field 1 B11 component <0.0>
 Field 2 B12 component <0.0>
 Field 3 B13 component <0.0>
 Field 4 B22 component <0.0>
 Field 5 B23 component <0.0>
 Field 6 B33 component <0.0>

SOLMOD

Set solvent modeling parameters

Field 1 Linear scaling constant for partial F calculated <1.0>
 term extracted as A part and B part from input BDF. The partial F calc will be multiplied by this factor before being added to the atomic F calculated.

Field 2 Exponential scaling constant, B. The partial F calculated is multiplied by EXP(-B*stol**2) before being added to F calc. <0.0>

Field 3 Linear scaling constant for the analytic solvent attenuation function, UK. <1.0>

Field 4 Experimental, exponential, scaling constant for the analytic solvent attenuation function, UB. Atomic scattering factors are multiplied by (1.0 - UK*EXP(-UB*stol**2)) prior to being used in the structure factor calculation. <0.0>

Field 5 Electron density plateau level to be used to model space not occupied by atoms. (?) <0.0>

STATS

Set resolution limits for R value calculations

Field 1-7 Resolution limits for determining R values for shells of reciprocal space. Maximum of seven shells; default one. Values supplied must be descending order d spacings in Angstroms.

WGTB

Field 1 Weight for temperature factor restraints, WB <1.0>
 applied weight in isotropic case = ((WB/sigma(B))**2 * (delta(B))**2)
 2 sigma of type 1 main-chain bond distances <1.0>
 3 sigma of type 2 main-chain angle distance <1.5>
 4 sigma of type 3 side-chain bond distance <1.0>

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EFLIM

Limit reflection included in refinement process

Field 1- Option control codes Option code

Function

Discard reflections based on the following criteria:

F relative < x.x <x.x=0.0>
 sin(theta)/lambda < x.x SMIN x.x <x.x=0.0>
 sin(theta)/lambda > x.x SMAX x.x <x.x=1.5>
 d maximum DMAX x.x <x.x=1000.>
 d minimum DMIN x.x <x.x=0.35>
 F(rel) < sigma(F(rel)) SIGM x.x <x.x=0.0>

note: Use either d or stol specs but not both.

TEST

Set up a survey of R values as a function of shift damping

Set up R testing for reflection subsets. The subsets are defined by the reflection count plus a constant modulo the frequency being zero. After the least-squares refinement structure factors are recalculated for the subsets in order to evaluate the efficacy of the shift damping and progress of the refinement.

Field 1 Sampling frequency for reflections to be included in the refinement and subsequent FC for R. <10000>
 Caution: If this number is set too low and fields 5 & 6 are large a great deal of time will be needed.

2 Sampling frequency for reflections to be excluded from the refinement but for which a subsequent structure factor R value will be calculated. This gives a stringent test of convergence, but caution is required or the number of reflections eliminated may be too great. <no>

3 Constant to offset from the beginning of sampling of included reflection subset. <0>

4 Constant to offset from the beginning of sampling of excluded reflection subset. <0>

5 Number of coordinate, x,y,z, shift steps to be tested. <1>
 Number of B steps to be tested. <1>

7-n Factors to multiply shifts for testing. <0.5>
 These are the same kind of factor as those in the DAMPSH line. e.g. x(test) = x(start) + factor*x(shift)
 The sum of fields 5 & 6 determines the value of n.

Set F relative scale factors

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5 sigma of type 4 side-chain angle distance <1.5>
 6 sigma of type 5 X-H bond <0.5>
 7 sigma of type 6 X-H angle <1.0>
 8 sigma of type 7 hydrogen-bond, metal, etc. <0.0>

WGTCCHI Field 1 Weight for chiral center restraints, WC <1.0>
 2 Sigma(chi) applied weight = ((WC)/sigma(chi))**2 <0.15>

WGTCOON Field 1 Weight for van der Waals contacts, WV <1.0>
 2 Sigma(vdv) applied weight = ((WV)/sigma(vdv))**2 <0.5>

The following fields may be used to supply parameters to modify the minimum "theoretical" van der Waals contact distance of four possible types which are defined and calculated in PROTIN and passed in file F. There are four parameters corresponding to:
 1) Atoms in which the relative position is determined by only one torsion angle.
 2) Two atoms in which two or more torsion angles are involved, multiple torsion contact.
 3) A possible (X....Y) hydrogen bond pair involving contacts between nitrogen oxygen pairs, but not N main - N main or O main - O main.
 4) A possible (X-H....Y) hydrogen bond.

3 Type 1 distance factor in Angstroms <-0.3>
 4 Type 2 distance factor in Angstroms <0.0>
 5 Type 3 distance factor in Angstroms <-0.2>
 6 Type 4 distance factor in Angstroms <0.0>
 Note: The distance factors must be negative amounts. They are used to adjust the ideal contact distances supplied from PROTIN in order to establish when a contact should be restrained.

WGTDIS Field 1 Weight for distance restraints, WD <1.0>
 The weight applied to a derivative is:
 ((WD/sigma(l))*ideal dist - actual dist)**2
 The following fields set sigmas for various types of distances. These types for possible i are:
 1) bonded distances (e.g. C alpha - C)

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2) angle distances (e.g. N - C)
 3) planar 1-4 distances (e.g. O 1-1 - C alpha)
 4) bond distances involving H (e.g. C alpha - H)
 5) angle distance involving H (e.g. H alpha - C)
 6) hydrogen bond, metal
 7) coordination, etc.

2 sigma of type 1 <0.03>
 3 sigma of type 2 <0.04>
 4 sigma of type 3 <0.05>
 5 sigma of type 4 <0.1>
 6 sigma of type 5 <0.08>
 7 sigma of type 6 <0.0>
 8 sigma of type 7 <0.0>

WGTPLA Field 1 Weight for plane restraints, WP <1.0>
 2 sigma of a plane <0.02>
 The weight applied to the derivative is:
 (WP/(sigma(P))**2

WGTTREF Field 1 Weighting scheme as 1 through 5 <1>
 2 A term for weights <4.0>
 3 B term for weights <0.0>
 Where:
 $Sd = A + B * (\sin(\theta) / \lambda - 0.1667)$
 Sa is the "sigma" used to calculate the structure factor weight = $1/Sa**2$
 scheme 1: Sa = Sd
 scheme 2: Sa = MAX(sigma(Frel), Sd)
 scheme 3: Sa = sigma(Frel) from input BDF
 scheme 4: Sa = A * sigma(Frel)
 scheme 5: Sa = SQRT(Sd**2 + sigma(Frel)**2)

WGTSHF Field 1 Weights to control excessive shifts <0.2>
 2 Positional shifts magnitude restraint, PDEL weight = (cell dimension/PDEL)**2 for a, b, & c <3.0>
 3 Individual temperature factor shifts magnitude restraint (not used if t.f. overall), BDEL. weight = $(1/BDEL)**2$ <0.1>
 4 Population parameter shifts magnitude restraint, QDEL. weight = $(1/QDEL)**2$
 The following six fields are for setting sigmas associated with positional and thermal parameter restraints. They set sigmas for "tight", "medium", and "loose" restraints respectively.
 5 Sigma for tight positional restraints <0.05>
 6 Sigma for medium positional restraints <0.5>

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```

6 Sigma for loose positional restraints <2.0>
7 Sigma for tight thermal restraints <0.5>
8 Sigma for medium thermal restraints <1.0>
9 Sigma for loose thermal restraints <0.0>

```

```

WGTSYM Field 1 Weight scale for non-crystallographic
symmetry restraints, WS. <1.0>
weight = (WS/SIGS)**2
WHAT IS SIGS????????????????????????????

```

```

WGTTOR Field 1 Weight scale for torsion angle restraints <1.0>
applied weight = ((Weight scale)/sigma(T))**2
2 Sigma(T) associated with a prespecified angle <15.0>
usually phi or psi of a regular secondary
structure.
3 Sigma(T) associated with a planar angle <3.0>
such as omega.
4 Sigma(T) associated with a staggered angle <15.0>
such as chi 1.
5 Sigma(T) associated with an orthonormal angle <20.0>
such as chi 2 of aromatics.

```

```

NOREF Field 1 - Atom identifiers for atoms which should not be
refined in any way. Continue on more NOREF lines
if need be.

```

EXAMPLE

Note that the program PROTON must have been run before PROLSQ may be run in order that the appropriate constraint information be available on file F for use in the refinement process.

```

PROLSQ CRAMB NCV 25 PCH ISO
PRINT ALIS 1
STATS 5.0 4.0 3.0 2.0 1.0
RTEST 5 23 1 1 4 3 .25 .45 .65 1.25 .35 .65 .95
END
FINISH

```

In this example the atomic parameters of crambin are to be refined with individual isotropic temperature factors. Twenty five conjugant gradient cycles will be used to establish the parameter shifts, the refined coordinates will be written to unitpch: as well as to BDF B. The PRINT line specifies that a listing of the shift information for all atoms shall be written to the print file. The STATS line directs that R values be calculated for

shells of resolution as shown. The RTEST line will cause every fifth reflection to be written to a scratch BDF on file G and every twenty third reflection to be written on file H. For both of these files the sampling frequency will begin with the first reflection. Once refinement is complete these scratch files will be used to calculate R values for the subset of reflections contained in them. Four structure factor calculations will be made damping the positional parameter shifts of the atoms by .25, .45, .65, and 1.25. Then three runs will be made damping the isotropic B shifts by .35, .65, and .95. Because of the 23 in the second field of the R test line the structure factors, and therefore, the R values for the excluded reflections will also be calculated. This test using excluded reflections is a sensitive measure of the success of a refinement. If the R value of the excluded sub-set does not track downward with that of the included reflections it may well be a signal of a problem with the model or that the restraints are too slack. That is that the distribution of the restrained parameters about their "ideal value" is too great; i.e. the energy is too high.

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PHACMP: Compare The Phases of Two Sets of Reflections

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SYNOPSIS

The purpose of PHACMP is to allow the comparison of the values of the reflection phases determined in two different ways for the same set of reflections. For example, the phases determined by an MIR calculation with those determined by a structure factor calculation. Input may either be from two phase sources on the same BDF or from two different BDFs. If two files are used, the reflection set must have been sorted into the same order by the use of the XTAL program SORTRF.

METHOD

This program calculates the mean difference and the weighted mean difference in the phase values, in degrees, for reflections. The estimated standard deviations in the differences are also calculated. The calculations are done as a function of "d" spacing, resolution, in equal volumes of reciprocal space. In addition, counts are made of the values of the phases in both sets as a function of angle. The number of resolution intervals and the range of angle for counts are under user control. Statistics are done only for those reflections which have established phases in both sets. Reflections for which the value of the phase is undetermined in either set are rejected.

All input phases from whatever source are reduced to degrees modulo 360. The differences in phases are defined as $\arccos(\cos(\pi-p_2))$ in order to place them all in the range 0 to 180 degrees. Note that the sign of the difference is lost by this method.

The use of this program requires the specification of the two different phase types to be compared. This is accomplished by the use of the PHASO1 and PHASO2 lines (PHA Source) which specify the source of phase information in terms of the types specified to exist in an XTAL BDF. The recognized mnemonics are:

- FC The calculated structure factor
Items n700 or n801 and n802 in lrrefl.; n is the data set number
- BEST The "best" Fourier coefficient* for the "native" or "parent" substance
Items 701 and 702 + (m-1)*4, in lrrefl:
where m is the alternate solution no.
- MOSF The "most probable" Fourier coefficient* for the "native" or "parent" substance
Items 701 and 702 + (m-1)*4, in lrrefl:
where m is the alternate solution no.
- DENS The calculated phase from density modification methods
Item n511 in lrrefl.; n is the data set number
- DIR Direct methods structure factor estimate
Items n631 + (m-1)*2, in lrrefl:

where n is the data set number and m is the alternate solution no.

In the case where the two sets are to be taken from separate binary data files with different compound identities provision has been made to supply the alternate compound identity in the PHACMP input line.

PRINTER OUTPUT

All printed output is at priority 3.

FILE ASSIGNMENTS

Reads BDF from file .AA1 for one or both phase sets
Optionally reads BDF from file .AA2 for second phase set. If the compound identity on the second file is different than that on the first file the correct one must be given in the PHACMP input line.

CONTROL LINES

PHACMP Program initiation line
* MAXHXL Maximum and minimum values of h, k, l, and $\sin(\theta)/\lambda$
* PHASO1 Source of first phase set
* PHASO2 Source of second phase set for comparison
END

* Indicates optional lines

PHACMP Program initiation line

Field 1	Compound ID	Function	Option code	Default
2-	List reflections modulo n	LIST n		<n=100>
	Take second set of phases from file .AA2	BDF2 s		<one file>
	If the compound ID on file .AA2 is different than that on file .AA1 it must be supplied following BDF2			<s=CPID of .AA1>
	Set range of phase values in each tally "bin" in degrees	BIN x		<x=22.5>
	Set number of equal volume shells in reciprocal space for statistics	SHEL n		<n=4>
	Do not copy the BDF for the second phase set from s.000 to s.aa2 before starting the calculation.	NOCO		<copy it>

Does not apply if BDF2 not given.

MAXHKL Scope of data to be scanned

Field	Quantity	Default
1	maximum h	<from file AAL lrdset:>
2	maximum k	<from file AAL lrdset:>
3	maximum l	<from file AAL lrdset:>
4	minimum sin(theta)/lambda	<from file AAL lrdset:>
5	maximum sin(theta)/lambda	<from file AAL lrdset:>
6	minimum h	<-1000>
7	minimum k	<-1000>
8	minimum l	<-1000>

PHASOL and PHASO2 Sources of phase information

Field	Function	Data set number	Option code	Default
1-	Phase source	DS n		<n=1>
	Calculated structure factors	FC		<FC>
	MIR "best" phases	BEST m		<m=1>
	MIR "most probable" phases	MOST m		<m=1>
	Density modification methods	DENS		<m=1>
	Direct methods	DIR m		<m=1>
	m is the solution number			

EXAMPLE

TITLE Test of PHACMP 19 Oct 1989
PHACMP TRIDI LIST 50 BDF2 TRIDI1 BIN 11.25 SHEL 10
PHASO1 FC
PHASO2 FC
END
FINISH

In this example the two binary data files TRIDI.000 and TRIDI1.000 will be used for the comparison of their calculated structure factor phases. Every fiftieth reflection will be listed. The statistics will be made in ten equal volumes of reciprocal space. Counts will be made of the number of reflections having phase values in "bins" from -5.625 to 5.625, 5.625 to 16.875, ..., and 343.125 to 354.375 degrees. Counts of differences will be made up through 180 degrees.

#####

REFOUT: Transfer Selected Reflection Data Into An ASCII File

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SYNOPSIS

The purpose of REFOUT is to allow the "punching" of selected reflection data from the standard BDF. For each reflection in a BDF an output line will be formed with h, k, l, and any other selected quantities present. Reflections for which any of the selected quantities are void will not be sent to the output file.

METHOD

This program runs in two modes. The "automatic" mode creates lines with h, k, l, |F(observed)|, |F(calculated)|, the phase in degrees, and sigma(F(relative)) as FORMAT(1X,I5,3X,2F12.3,F12.1,F12.3). All input phases from whatever source are reduced to degrees modulo 360.

In the "user controlled" mode h, k, and l are written out in the same form, but all other quantities are chosen by use of an IDNUM input line. This line accepts ID numbers as described in the appendix on the binary data file for packets of record lrrrefl. For example F relative for the "parent" derivative is ID number 1304. The format of each quantity specified in the IDNUM input line may be specified, in XTAL notation, in a FORMAR input line. This line is optional if the format shown above is satisfactory. Finally, an optional SCALEQ input line allows the selected quantities to be multiplied by a supplied factor. The default is 1.0 for the SCALEQ quantities.

PRINTER OUTPUT

All printed output is at priority 3.

FILE ASSIGNMENTS

Reads BDF from file .AAL
Writes line images on .PCH

CONTROL LINES

REFOUT Program initiation line
*# IDNUM lrrrefl: ID numbers for selected quantities
* FORMAT Format, in XTAL notation for each selected quantity
* SCALEQ Multiplying scale factors for each selected quantity
END

* Indicates optional lines
 # This line switches program from "automatic" to "user controlled"

REFOUT Program Initiation line

Field 1 c pound ID
 2- Function in "automatic" mode Option code Default
 List first n reflections LIS n <n=20>
 Scale F relative SCA x.x <x.x=1.0>
 Data set to be "punched" DSE n <n=1>

IDNUM Reflection record ID numbers for "user controlled" output
 h, k, and l are always written by default. There are no
 other defaults.

Field Quantity
 1-n Chosen ID numbers. First void field terminates the scan.

FORMAT Format of data for output
 h, k, and l are always 1X,1I5 by default. The first void
 field, or a field less than 10001, terminates the scan.

Field Quantity Default
 1 First beyond hkl <311232.>
 2 Second <431232.>
 3 Third <551212.>
 4 Fourth <671232.>
 5 - Fifth and beyond <uninitialized>

Note: An XTAL format consists of a real number of the
 form cccwdt. Where: ccc is the ending column for
 the item, ww is the width of the item, d is the number
 of places after the decimal point for reals or the minimum
 number of digits for integers; t is the type of
 output as 1 for E, 2 for F, 3 for I. For example the
 formats for h, k, and l are 60513., 110513., and 160513..

SCALEQ Scale to be applied to corresponding fields in FORMAT line.
 Void fields are skipped as are fields less than |0.0001|.

Field
 1-n Function Default
 Multiplicative scale factor <1.0>

EXAMPLE

TITLE TEST OF REFOUT 1 Dec 1989
 REFOUT SALY
 IDNUM 1600 501 1309
 FORMAT 250742. 351031. 400513.

SCALEQ 1.5 1000. 10.
 END
 FINISH

In this example the normalized structure factor, E1 - item 1600, scaled by
 1.5 is to be written F7.4 ending in column 25; the first interpolated
 scattering factor, f1 - item 501, scaled by 1000. is to be written E10.3
 ending in column 35; and the scale group number, item 1309, scaled by 10
 is to be written I5 ending in col 40. If ending columns before 16 had
 been specified h, k, and/or l would have been overwritten. Take care as
 very little screening of these format numbers is made by this program.

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